The Effect of Low-Molecular-Weight Heparin on Microvenous Thrombosis in a Rat Model

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**Objective:** To assess the impact of a low-molecular-weight heparin sodium, dalteparin sodium, on a thrombogenic microvenous anastomosis, using a randomized, blinded animal model.

**Methods:** Using male Sprague-Dawley rats, 70 IU/kg of dalteparin sodium (for the treatment group) or isotonic sodium chloride solution (for the control group) were administered subcutaneously in a blinded randomized fashion. Using microsurgical techniques, the femoral venous pedicle was isolated bilaterally. A tuck anastomosis was then performed on each side. Vessel patency was assessed periodically for 3 hours using a strip and refill test. Patency or thrombosis was confirmed by cutting the vessel proximal to the anastomosis and examining the lumen for thrombus.

**Results:** A total of 58 venous tuck anastomoses were performed. There was no difference in bleeding complications between the treatment and control groups. The control group had a thrombosis rate of 50%, and the treatment group had a thrombosis rate of 60%. The $\chi^2$ analysis does not indicate a statistical difference between these 2 groups ($P = .59$).

**Conclusion:** Low-molecular-weight heparin, at standard therapeutic dosing, may not provide an adequate antithrombotic effect to prevent anastomotic thrombosis in free tissue transfer.

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MICROVASCULAR SURGERY has developed into an integral part of head and neck reconstruction. The ability to transfer a wide range of tissues such as fasciocutaneous, myocutaneous, myofascial, osseous, and osseocutaneous flaps has given surgeons the ability to address considerable lesions and the resultant defects. Free tissue transfer has reached high levels of success in terms of flap survival. Most studies report an overall survival rate of 95%. Although numerous factors contribute to flap failure, thrombosis of the microvascular anastomosis is considered to be the major cause of failure. The consequences of vessel thrombosis are severe and cause significant morbidity and even mortality.

Thrombosis of the pedicle, usually the vein, has been reported to occur in 3% to 15% of microvascular anastomoses. Thrombus formation in the high-flow arterial system is considered to be most dependent on platelet effects whereas thrombosis in the venous system is more dependent on tissue factors activating the coagulation cascade. In hopes of decreasing anastomotic thrombosis and improving overall success, there have been several pharmacologic attempts to prevent thrombus formation. Unfractionated heparin sodium, dextran, and aspirin have been evaluated; however, none of these pharmacologic agents has eliminated thrombosis without causing additional risk and morbidity. Therefore, investigations for the ideal antithrombotic agent continue. The aim of this study was to investigate the effect of a low-molecular-weight heparin on the venous anastomosis using a rat tuck model.

**METHODS**

After institutional review board approval, male Sprague-Dawley rats weighing 250 to 300 g were used. They were cared for under the supervision and protocols of the institution's animal care facility. In a randomized blinded fashion, 70 IU/kg of dalteparin sodium (Fragmin; Pharmacia Corporation, Kalamazoo, Mich; 70 IU/kg is the manufacturer’s recommended dose for preventing catheter thrombosis) (for the treatment group) or isotonic sodium chloride solution (for the control group) were administered subcutaneously. After 3 hours, each rat was anesthetized with an intramuscular injection of ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (10 mg/kg). Once adequate anesthesia was achieved and confirmed with pinch testing, a transverse abdominal incision was made, and bilateral femoral neurovascular bundles were exposed. Using the
operating microscope and microsurgical techniques, each femoral vein was isolated from the neurovascular bundle and stripped of excess adventitia. While isolated, a 2-vessel atrumatic vessel clamp was placed. A venotomy was then created by incising 180° of the vessel lumen. Isotonic sodium chloride solution was used to irrigate and clear each end of the clamped vessel.

As originally described by Stepnick et al and modified for the rat vein by Laxmeesh Nayak, MD (unpublished data, 2004), a tuck procedure was performed (Figure). Using a 10-0 nylon suture, a tuck anastomosis was created by first entering the proximal cut end of the vein and then exiting slightly more distally on the same proximal segment of the vein approximately 1 mm from the venotomy edge where the suture rested extraluminal. It was then reintroduced through the lumen of the distal segment of the venotomy, exiting through the distal segment of the vessel. A single square knot was thrown to close the venotomy, allowing the vein to refill. The same procedure was then performed on the opposite side.

Vessel patency was assessed periodically for 3 hours using a strip-and-refill test in which the vessel was gently occluded anatomically proximal to the anastomosis while a second forceps was used to strip the venous blood in the more proximal portion of the vein. The second forceps was kept in place at the proximal end of the vein to prevent retrograde filling while the remaining forceps was released. This allowed blood to flow across the anastomosis and refill the stripped segment. If blood flowed across the anastomosis, it was considered patent. If there was no refill, the vessel was considered thrombosed. Finally, patency or thrombosis was confirmed by cutting the vessel proximal to the anastomosis and examining the lumen for thrombus. Completion was considered when thrombosis occurred in each vessel or 3 hours passed without thrombus formation. Finally, the rats were euthanized, while anesthetized, with a lethal intravascular injection of pentobarbital sodium.

RESULTS

One rat in the control group died of complications from the anesthesia prior to any incisions. A total of 58 venous tuck anastomoses were performed. During dissection, 2 rats were found to have a small hematoma in the inguinal region; one received treatment with dalteparin, and the other received the control treatment. In both cases the hematoma was found to be contralateral to the injection site and did not prevent performance of the tuck procedure.

The control group had a thrombosis rate of 50% (14/28), and the treatment group had a thrombosis rate of 60% (18/30). The χ² analysis does not indicate a statistical difference between these 2 groups (P = .59). As in other studies, nearly all vessels that thrombosed did so in the first 60 minutes. In the treatment group, there were 2 vessels that were noted to be tenuous at each evaluation but that did not thrombose until 2 hours and 20 minutes and 2 hours and 50 minutes had passed, respectively.

Because of the high morbidity rate associated with free-flap failures, investigations into techniques and pharmacologic agents that may reduce the rate of anastomotic thrombosis continue. Historically, several agents have been used, including unfractionated heparin, dextran, and aspirin. Each has shown some therapeutic benefit, but each has a significant risk of additional complication.

Therefore, the search continues for the ideal agent, one that should eliminate thrombosis at the anastomosis without affecting coagulation parameters that would predispose to bleeding complications such as hematoma. It should not have additional systemic effects, such as volume overload, or renal adverse effects that could lead to further morbidity. The agent should be easily administered at a reasonable cost. Low-molecular-weight heparin shows particular promise. It was initially recognized in the treatment and prevention of deep vein thrombosis and is now being applied in other medical and surgical settings. Research into the impact of low-molecular-weight heparin on microsurgical anastomoses and small artery and vein thrombosis has been promising.

Zhang and Weislander used a trauma model performing arteriomy and venotomy and intimectomy to assess the impact of intravenously administered low-molecular-weight heparin, dalteparin. They found statistically significant differences in rate of patency and weight of thrombotic material. Low-molecular-weight heparin and unfractionated heparin were equally effective in preventing vein thrombosis; however, unfractionated heparin caused increased bleeding. Miyawaki et al created a venous congestion flap model for the rabbit. They found flap circulatory territory to be significantly greater for low-molecular-weight heparin, suggesting greater patency in microvasculature. In an effort to assess both microvascular patency and flap survival, Ritter et al created a rat flap model with a venous anastomosis. Administering intravenous enoxaparin sodium, they found statistically significant increases in patency and flap survival compared with controls. They found increased survival for the low-molecular-weight heparin group compared with the unfractionated heparin group as well. Last, they measured anti–factor Xa activity and reported a significant increase in activity for low-molecular-weight heparin over the unfractionated heparin.

This evidence is promising for low-molecular-weight heparin. To further assess its clinical signifi-
cance and potential for having an impact on thrombosis in human microvascular surgery, we used a thrombo- 

genic microvenous anastomosis tuck model. This model is 
efficient for creating a clinically active and reproduc-
tively thrombogenic source, and it allows for testing of phar-
macologic agents in a clinically relevant manner.

Previous work in our laboratory\(^1\) demonstrated no 
statistically significant decrease in thrombosis using the 
low-molecular-weight heparin enoxaparin in a microar-
terial tuck model. Explanations for this include possible 
dosing below effective antithrombotic levels, inade-
quate time to thrombosis formation (2 hours of obser-
vation), or the mechanism of thrombosis in an arterial 
tuck model may be more dependent on platelet aggrega-
tion as opposed to tissue factor.

Given the potential for greater impact by tissue fac-
tors on thrombus formation in the microvenous system, 
investigation of low-molecular-weight heparin in a venous 
model remained prudent despite the initial failure to show 
a difference in the arterial model. Based on the previous 
experience, several changes were made.

Dalteparin was used instead of enoxaparin because of 
reported differences between low-molecular-weight he-
parin.\(^12\) Dalteparin seems to have the most optimal pro-
file for preventing thrombosis without additional risk of 
blooding. Vessels were monitored for a total of 3 hours 
in this study (increased from 2 hours). Also, a higher 
equivalent dosage of dalteparin was used rather than of 
exoxaparin, as in the previous experiment, to allow greater 
antithrombotic levels to be reached.

Despite these changes, there was not a demonstrable 
difference between the control and treatment groups. With 
the exception of 2 vessels in the treatment group, all 
thromboses still occurred within the first 60 minutes. The 
consistent results between the arterial and venous mod-
els may suggest that low-molecular-weight heparin does 
not provide adequate antithrombotic effects at the mi-
crovascular anatomic site. The platelet mediated ef-
fects in thrombus formation may dominate to such a de-
gree in all small vessels, artery and vein, that more targeted 
and powerful antiplatelet agents are necessary to have an 
impact on thrombosis rates.

Similarly, a dosing issue may be present. Other stud-
ies\(^8-10\) showing potential for low-molecular-weight he-
parin have typically used intravenous administration. Per-
haps this allows for greater antithrombotic activity, 
although pharmacologically there should not be a dif-
ference. Higher dosages of low-molecular-weight he-
parin have been used in some previous studies\(^9\) and in other 
clinical settings such as treatment of deep vein throm-
bosis and myocardial ischemia. However, the dosage used 
in this study is approximately 2 times higher than the 
recommened deep vein thrombosis prevention dosage. 
Other models in which low-molecular-weight heparin 
have been effective, such as the trauma model, may 
have a greater degree of tissue factor activation 
because of the nature of the model. Similarly, various flap 
models that do not create a thrombogenic source may 
activate the coagulation cascade in a different manner com-
pared with the tuck model.

In conclusion, this study investigated the potential 
antithrombotic effect of dalteparin in a microvenous tuck 
anastomosis model. There was not a statistically signifi-
cant difference between the treatment and control groups. 
The treatment group did not show any signs of adverse 
effects; in particular, there was no increased incidence 
of bleeding. These data, when considered with previ-
ously published data on the effect of enoxaparin on the 
microarterial tuck anastomosis model, suggest that low-
molecular-weight heparin may not provide an adequate 
antithrombotic effect to prevent anastomotic thrombo-
sis in free tissue transfer.

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